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The title of the invention has been changed (as directed per technical review by OEB, A-III, 7-3)

(54) Cosmetic Use of N-acetylated or N-propionylated Derivatives of Proline, Hydroxyproline and/or of a Mixture of Amino Acids obtained by the Hydrolysis of Collagen.

(57) This invention relates to the application in cosmetic products of N-acetylated or N-propionylated derivatives of proline, hydroxyproline and/or a mixture of amino acids obtained by collagen hydrolysis as well as the salts of these derivatives.
The invention also relates to cosmetic compositions containing said derivatives.

COSMETIC APPLICATION OF DERIVATIVES OF PROLINE, HYDROXYPROLINE AND/OR A MIXTURE OF AMINO ACIDS OBTAINED BY THE HYDROLYSIS OF COLLAGEN

Description

This invention relates to the application in cosmetic products of N-acetylated or N-propionylated derivatives of proline, hydroxyproline and/or a mixture of amino acids obtained by collagen hydrolysis, the salts of these derivatives, as well as to compositions containing said derivatives.

N-acylated derivatives from certain amino acids have been described earlier in cosmetic applications. For example, French patent 2 503 153 addresses the use, especially in the realm of cosmetics, of amino acid derivatives obtained by combining the butyric chain with the acylable functions of proline, hydroxyproline and hydroxylysine. These derivatives have good skin-penetrating properties due to their hydrosoluble characteristics and the nature of their lipidoprotidic structure. Their cosmetic role is based on an acidification of the skin. It is a known fact that the physiologic stability of the skin is in large measure a function of the acidobasic balance of the surface layer of the stratum corneum. That is why these acidifying products are present in numerous cosmetic compositions.

The applicant has found, however, that those butyrylated derivatives have a strong odor, making them unsuitable for cosmetic applications. Indeed, one of the most important criteria for cosmetic products is the absence of any unpleasant odor.

Moreover, some of those butyrylated derivatives are unstable: they turn black over time.

To avoid these drawbacks, the applicant has arrived at a cosmetic application of amino acid derivatives that do not produce an unpleasant odor, are stable over time and offer good tissular penetration even though they do not contain an aliphatic chain.

The applicant has also made the surprising discovery that these derivatives, in addition to the above benefits, offer a remarkable oxygenating and regenerating effect on cutaneous cells.

It is a known fact that the metabolic action of a substance, and thus its cosmetic potential, can express itself at the cutaneous level by an increase in the oxygen consumption which contributes to cell regeneration.

Then too, these derivatives help to significantly reduce the enzymatic attack on the elastins by elastase. It follows that these derivatives can be viewed as being supportive in terms of skin elasticity.

One objective of this invention is therefore the introduction in cosmetic products of compositions that have no unpleasant odor, are stable over time, penetrate the skin, display a significant ability to oxygenate and regenerate cutaneous cells and allow the skin to retain its elasticity.

The invention achieves this in cosmetic products by applying N-acetylated or N-propionylated derivatives of proline, hydroxyproline and/or a mixture of amino acids obtained by collagen hydrolysis as well as the metal or organic base salts of these derivatives.

Other features and advantages of the invention will be more clearly understood based on the following description.

The N-acetylated and N-propionylated derivatives of proline and hydroxyproline can be prepared, in conventional fashion, by themselves, by acylation in an aqueous or organic medium of amino acids, with the aid of acetic-acid-activated derivatives and with propionic acid. The activated derivatives include for instance acetic or propionic anhydrides or acetyl or propionyl chlorides. The process is typically performed at a temperature between 0 and 100°C.

The N-acetylated and N-propionylated derivatives thus obtained may be purified by crystallization or by chromatography.

Within the scope of this invention the term collagen refers to animal proteins from various sources contained in the basic support tissue.

The collagen hydrolysis can take place in traditional fashion by heating the collagen at temperatures between 60 and 130°C in an aqueous acid medium. The acidification of that medium can be obtained by means of strong acids such as sulfuric acid or chlorohydrous acid.

The amino acid mixture produced can then be neutralized using a strong base such as soda or potassium. In a final step, that amino acid mixture in an aqueous solution can be acylated using the same process as that employed in acylating the proline and the hydroxyproline with the aid of the afore-mentioned acetic and propionic acid activated derivatives.

The N-acetylated or N-propionylated amino acid mixture may be purged of any impurities for instance by distillation and rinsing in water in a vacuum. That purification process can take place at temperatures between 30 and 60°C and preferably between 40 and 50°C.

This invention also covers the salts of the derivatives of N-acetylated or N-propionylated proline, hydroxyproline or mixtures of amino acids obtained by collagen hydrolysis. These salts can be prepared by a reaction of the said N-acetylated or N-propionylated derivatives with organic or inorganic bases or with metallic derivatives.

Organic or inorganic bases within the scope of this invention include potassium, soda, lime, magnesium, ammonia; triethanolamine, diethylamine, morphine, lysine, histidine, arginine, ornithine, choline.

With regard to salts obtained by reaction with metal derivatives, choices include the salts of zinc, aluminum, copper, cobalt, iron and manganese.

Within the scope of this invention, the N-acetylated or N-propionylated derivatives of proline, hydroxyproline and/or a mixture of amino acids obtained by collagen hydrolysis as well as the salts of these derivatives are employed in the formulation of cosmetic compositions. These cosmetic compositions may be in the form of creams, milks, foams, aerosols, gels, sticks, oils, emulsions, soaps as well as aqueous or hydroalcoholic lotions.

In these cosmetic compositions the above N-acetylated or N-propionylated derivatives or the salts of these derivatives are contained in proportions of the order of 0.1 to 20% and preferably from 0.5 to 10% by weight in relation to the total weight of the composition.

The cosmetic compositions within the scope of this invention are stable, free of any unpleasant odors while conferring to the cutaneous cells a remarkable oxygenating and regenerating ability, displaying their strong cosmetic potential and allowing the skin to retain its elasticity.

The following are intended to serve as examples only and are not to be considered as limiting in terms of the domain and the spirit of the invention.

Examples 1 to 4:

These examples relate to the cosmetic compositions according to the invention. The active ingredients in these compositions are N-propionyl/L hydroxy-4 proline, N-propionyl L proline or the N-propionylated derivative of the amino acid mixture obtained by collagen hydrolysis.

Example 1

Regenerative Cream

Composition:

A

Tefose 500® 10 g
Glycol propylene depelargonate 5 g
Liquid vaseline oil 5 g
Cetyl alcohol 3 g

B

Methyl parahydroxybenzoate 0.15 g
Sodaic methyl parahydroxybenzoate 0.15 g
Active ingredient 10 g
Sodium acid carbonate a/a for pH 5
Water a/a for 100 g
Perfume 0.1 g

Cream preparation procedure

A and B are separately heated to 70°C.

Next, B is mixed into A with moderate agitation. The mixture is cooled to 30°C and perfume is added.

This regenerative cream is intended for facial care and has an anti-wrinkle effect.

Example 2

Massage cream

Composition:

A

Cetasal 15 g
Labrafil 2130 CS 5 g
Clear vaseline 3 g
Silicone oil 1 g
Parlearn 2 g
Methylparahydroxybenzoate 0.1 g

B

Sorbitol 2 g
Sodaic methylparahydroxybenzoate 0.1 g
Active ingredient 10 g
Sodium acid carbonate a/a for pH 5
Water a/a for 100 g
Perfume 0.1 g

Cream preparation procedure

A and B are separately heated to 70°C. Next, B is mixed into A. The mixture is cooled to 30°C and perfume is added.

This massage cream serves to prevent weals or to improve their appearance.

Example 3

Body milk

Composition:

A

Liquid vaseline oil 10 g
Isopropyl palmitate 4 g
Sunflower oil 5 g
Cetyl alcohol 1 g
Tween 60 2.6 g
Span 60 2.4 g

B

Kathon CG (solution) 0.1 g
Active ingredient 5 g
Sodium acid carbonate a/a for pH 5
Water a/a for 100 g
Perfume 0.1 g

Milk preparation procedure

A and B are separately heated to 70°C. Next, B is mixed into A with rapid agitation. The mixture is cooled to 30°C and perfume is added.

This milk is applied daily and serves to maintain and improve the elasticity of the skin.

Example 4

Gel mask

Composition:

Chamomile glycol extract	7 g
Glycerol	10 g
Carbopol 940	1 g
Triethanolamine	a/a for pH 6
Active ingredient	5 g
Kathon CG (solution)	0.1 g
Colorant SQ	
Water-soluble perfume	0.3 g
Water	a/a for 100 g

Gel mask preparation procedure:

The Carbopol is dispersed in ¾ of the total amount of water. The remainder of the water and all components of the formula except for the triethanolamine are made into a solution.

Next, this mixture is slowly addedt o the dispersed Carbopol, then neutralizedt with the triethanolamine.

This gel mask can be applied in a thick layer and left on the skin for 20 minutes. After rinsing and quickly drying the skin, an emollient cream is applied.

Examples 5 to 9

These examples will show the ability of the N-acetylated and N-propionylated derivatives to oxygenate and regenerate cutaneous cells.

The techniques employed in the tests on which these examples are based involved oxygen pressure measurements, i.e. the "oxygraphic method". These tests were performed on hepatic mouse cells since it is an established fact that oxygen consumption measurements on these cells actually reveal the oxygen consumption of cutaneous cells and thus their regenerative ability.

The tests were carried out as follows: For the oxygen consumption measurement a BECK-MANN 39550 oxygen electrode was used, which is a Clarke-type glass electrode that permits the tracking of variations in the dissolved oxygen concentration. The electrode was equipped with a test cell consisting of a cylindrical glass tube; the electrode thus equipped was immersed in a water bath thermostatically controlled between 30 and 50°C.

3.6 ml of Ringer's fluid containing crushed mouse liver was introduced in the cell. The con-centration of crushed mouse liver was 30 mg per ml of Ringer's fluid. The oxygen consumption was measured over a 10-minute period, after

which an amount of the product to be tested, diluted in Ringer's fluid, was added to the cell.

A new, 10-minute oxygen consumption measurement was performed.

Adding the product per this invention to be tested produced a variation in the oxygen consumption. That variation was determined along the following formula:

$$\text{var.} = \frac{B - A}{A} \times 100$$

where

- var. is the percentage of variation in the oxygen consumption,

A is the value in ppm of the oxygen consumption at the time the product to be tested was added,

B is the value in ppm of the oxygen consumption 10 minutes after the test product was added.

The results obtained with examples 5 and 6 are shown in the following table:

Table I

Exple	Product tested	Concentration of test product in the cell (% by volume relative to the total volume in the cell)	Variation in the oxygen consumption
5	C_3Co	1%	220%
6	C_3Pro	1%	78%
7	C_3HP	1%	110%
	C_3HP	1.43%	135%
9	C_2HP	1%	42%

In this table: C_3Co is the N-propionylated derivative of the amino acid mixture obtained by collagen hydrolysis.

C_3Pro is the N-propionyl L proline

C_3HP is the N-propionyl L hydroxy-4 proline

C_2HP is the N-acetyl L hydroxy-4 proline.

These examples clearly show that the addition of the compositions according to the invention promotes cell oxygenation.

Example 10:

The tests for this example have shown the protective ability of elastin vis-à-vis the elastase, provided by the compositions according to the invention.

These tests were based on the principle of elastate diffusion in a 1% agar gel in which insoluble orcein-dyed elastin was dispersed.

After 48 hours of incubation in an oven at 37°C

the surface of the clear zones corresponding to the enzymatic digestion zone was measured.

The tests were performed on a reference gel (test A) and on gels containing compositions according to the invention, specifically:

- Test B: N-propionyl proline (C_3Pro)
- Test C: N-propionyl L hydroxy-4 proline (C_3HP)
- Test D: N-propionylated derivative of the amino acid mixture obtained by collagen hydrolysis (C_3Co)

The gels were produced as follows:

- gel in test A:

0.25 g of Agarose Prolabo® powder was buffered with TRIS at pH 8.3 (TRIS pH 8.3 is prepared from a TRIS-$CaCl_2$ mixture whose pH is adjusted to the desired value using HCl 0.1 N)

The mixture was placed in a boiling water bath for 15 to 20 minutes until a clear solution was obtained. That solution was then kept in the water bath at 40°C, then mixed with 0.0375 g Sigma E-1500 orcein-elastin. 20 g of that mixture was separated into two Petri dishes 90 mm in diameter.

- gels in tests B, C and D;

0.25 g of Agarose was mixed with 18 ml TRIS pH 10.4 buffer to which 25 ml water was added. The mixture was placed in a boiling water bath, then held at 40°C as above.

Next, at 40°C, 25 g of the gel thus produced was mixed with 0.0375 g of orcein-elastin and either 0.125 g C_3Pro (test B) or C_3HP (test C) or 0.25 g C_3Co (test D).

The pH of the gel was now 8.3.

Next, 20 g of each mixture was separated into two Petri dishes 90 mm in diameter.

Elastase deposition

In each gel obtained as described above a recess 3mm in diameter was punched out. 5 µl E-1250 porcine pancreatic elastase (activated 73 U/mg protein) was placed in each recess.

Next, the Petri dishes were kept at 37°C for 48 hours, then the surface of the digestion zone around each recess was measured.

That zone was a white spot on a blue base.

The results of these tests are shown in table II below:

Table II

Test	Product tested	Surface in mm^2	Δ %
A	-	300	-
B	C_3Pro	213	-39%
C	C_3HP	226	-24.66%
D	C_3 Co	201	-33%

In the table, Δ % represents the decrease of the attack on the elastin by the elastase.

That value is obtained via the following formula:

$$\Delta\ \% = \left(\frac{S1}{S2} - 1\right) \times 100$$

where S1 is the surface of a digestion zone in a gel containing a composition according to the invention and S2 is the surface of a digestion zone in the reference gel.

Patent Claims

1. Use in cosmetic products of N-acetylated or N-propionylated derivatives of proline, hydroxy-proline and/or a mixture of amino acids obtained by collagen hydrolysis, as well as of the metal salts or organic bases of said derivatives.
2. Cosmetic compositions, characterized in that they contain derivatives of N-acetylated or N-propionylated proline, hydroxyproline and/or a mixture of amino acids obtained by collagen hydrolysis or of the metal salts or organic bases of said derivatives.
3. Cosmetic compositions as in claim 2, characterized in that they contain from 0.1 to 20% by weight, relative to the total weight of the composition, of the said N-acetylated or N-propionylated derivatives or their salts.
4. Cosmetic compositions as in claim 3, characterized in that they contain from 0.5 to 10% by weight, relative to the total weight of the composition, of the said N-acetylated or N-propionylated derivatives or their salts.
5. Cosmetic compositions as in one of the claims 2 to 4, characterized in that they are in the form of creams, milks, foams, aerosols, gels, sticks, oils, emulsions, soaps, aqueous or hydroalcoholic lotions.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 40 1902

DOCUMENTS CONSIDERED PERTINENT

Category	Citation of document with indication, where necessary of the parties concerned	Pat. claim concerned	APPLICATION CLASS'N (Int. Cl⁴)
Y	FR-A 2 237 616 (PROCTER & GAMBLE) * in its entirety *	1-5	A 61 K 7/48 A 61 K 7/06
Y	FRA-A-1 520 356 (BUREAU D'ETUDES TECHNIQUES) * in its entirety *	1-5	
A,D	FR-A-2 503 153 (MORELLE et al.) * in its entirety *	1-5	
A	EP-A-0 197 510 (KOKEN CO.) * in its entirety *	1,2,5	
A	EP-A-0 197 506 (KOKEN CO.) * in its entirety *	1,2,5	
A	INTERNATIONAL JOURNAL OF COSMETIC SCIENCE, Vol. 8, 1986, pages 73-80; M.C. MARTINI et al.: "Influence of butyrylhydroxyproline on the development of fibroblasts in culture" * Pages 73-80 *	1-5	
			TECH. FIELDS RESEARCHED (Int. Cl⁴) A 61 K

This report has been prepared for all claims

Place of search	Date search completed:	Examiner
THE HAGUE	02 Dec 1988	FISCHER J.P.

CATEGORY OF DOCUMENTS CITED

(standard boiler plate)